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INSUSCEPTIBILITY OF MAN TO INOCULATION WITH BLOOD FROM MEASLES PATIENTS

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INTRODUCTION

In congested communities the sanitarian experiences greater difficulty in the control of diseases of the respiratory tract than in the case of those infections in which the portal of entry is by way of the alimentary canal. From the standpoint of epidemiology, measles should certainly be classified with the respiratory diseases; it takes a prominent place among the most highly communicable infections, being excelled, perhaps, only by pneumonic plague. In the mobilization of recruits even the uncomplicated cases of measles may produce a high non-effective rate; in addition to this disadvantage the serious pulmonary complications of the disease render desirable the development of a protective immunization against the primary infection. Investigations for this purpose would require the inoculation of human subjects on a basis which is necessarily more or less experimental in character. Indeed, of the serious infections of man, the virus of measles like that of scarlet fever and yellow fever * can be definitely identified only by the inoculation of susceptible human individuals. During the recent military emergency, a need and an opportunity arose for the investigation of measles such as would not occur in civil life. Accordingly, Colonel Frederick F. Russell recommended to General William C. Gorgas and to General Meritte W. Ireland the study in human volunteers of the question of prophylactic inoculation against measles. In time of need the individual soldier was found ready and willing to offer his services and to accept such risk as was inherent in these inoculations. A precedent for this type of work has been firmly established; the conditions in army life seem especially favorable for the development of preventive medicine as evidenced by the progress in the control of yellow fever, of typhoid and of trench fever.

* In a recent communication Noguchi reports the discovery of the etiologic agent of yellow fever.[1]

LITERATURE

Human Inoculations.—Accurate records of the experimental production of measles in man are meager in the extreme. The easy recognition of the disease by its clinical symptoms would seem to render of interest the investigations conducted before the bacteriologic era. For example, Mayr[2] in 1852 reported the occurrence of measles after an incubation period of two weeks in two of three subjects inoculated on the nasal mucous membrane with nasal secretions of a patient. In another experiment, Mayr reports that mild but typical attacks were produced in each of six subjects by introducing into the skin the scrapings from a morbillous lesion. The incubation period is not stated; no mention is made of any precautions to avoid spontaneous infection.

Recently Herrman[3] (1915) inoculated 40 infants under five months of age on the nasal mucous membrane with nasal secretions from cases of measles. This was done, not with the

idea of producing the disease but with the expectation of establishing an immunity, taking advantage of the relatively high insusceptibility of infants to measles in the first few months of life. None of these 40 infants developed any definite symptoms. About 1 to 2 years later, 4 of these children came in intimate contact with measles cases and two were reinoculated without developing the disease.

The first experimental inoculations of blood were performed by Hektoen[4] in 1905; this observer conducted two independent tests and reported an attack of measles in each instance. Blood for inoculation was taken during the eruptive stage and was incubated before injection, for one day at 37° C. in ascitic broth (1 part ascitic fluid to 2 parts meat infusion broth). In one patient the specimen of blood was withdrawn 6 hours and in the other 30 hours after the eruption had appeared. Moderate quantities (3 c. c. and 2.5 c. c.) were inoculated into flasks of ascitic broth (50 and 75 c. c. amounts). After incubation for 24 hours, this mixture was apparently sterile; there was no evidence of secondary contamination or of any multiplication of the virus of measles as indicated by the gross appearance, by microscopical examination and by the subsequent results of sub-inoculation on a variety of media. Accordingly subcutaneous injections were made (4 and 5 c. c.) into two volunteers and apparently a mild but typical attack of measles developed in each case after an incubation period of about two weeks. This volume of broth culture represents a calculated quantity of blood serum of approximately 0.1 c. c. All viruses known to multiply in culture media produce either macroscopic or microscopic evidence of their growth; both manifestations appear in the vast majority of cases. It would seem from these results, therefore, that a very small amount of measles blood is infective and that it retains its virulence for at least a day *in vitro* at incubator temperature. Such a conclusion is of far-reaching importance. In the investigation of a disease of unknown etiology a great advantage is achieved by the isolation of its virus in a relatively concentrated form uncontaminated by other micro-organisms; one is then in possession of suitable material for the investigation of fundamental features such as immunization of the natural host, transmission to other species and studies in morphology and cultivation. Practically the entire superstructure of modern experimental work in measles rests on these two experimental cases. One may call attention more especially to the reported transmission of the disease to monkeys by inoculations of blood and the isolation of a micrococcus from the blood stream by Tunnicliff.[5]

Inoculation of Monkeys.—Of the recent investigators, Anderson and Goldberger[6] were the first to report the successful inoculation of monkeys with measles. Two of their conclusions are of especial interest in connection with the data reported in this paper, namely: (1) that the blood of early cases is infective and (2) that the virus of the disease as it occurs in the blood is capable of passing a Berkefeld filter.

DESCRIPTION OF EXPERIMENTAL WORK

In arranging the preliminary protocol of the following work, the reported transmission of measles by the inoculation of blood was accepted as a basis for the first series of injections, arrangements being made to conduct a control for confirming the findings concerning the infectivity of the blood. In order to provide a secure foundation for the more elaborate and time-consuming experiments it was expected in the course of these investigations to obtain information concerning the constancy with which the blood of individual cases is infective, the minimal quantity which suffices to produce the disease and also the extreme limits during which the virus may be demonstrated in the blood. Inasmuch as a very favorable control for the first series of inoculations failed, the question of the infectivity of the blood in measles was accepted as the primary problem; the ultimate object of these investigations consists in determining whether a method of active immunization can be developed, without producing a serious reaction. Injections of blood from patients in various stages of the disease have been made into supposedly susceptible individuals but no symptoms of measles appeared in any instance. Two individuals who had been injected with blood were subsequently inoculated on the mucous membrane of the upper respiratory tract with nasal secretions of an early case of measles but no symptoms developed. There is some reason to believe that these individuals were protected by the preliminary injection of blood.

Arrangements and Preparations for Inoculations.—These investigations were conducted at Camp Devens, Mass., and at Camp Meade, Md. Camp Devens covers an area of approximately 16½ square miles with a normal capacity for 35,000 to 45,000 troops. The Base Hospital is situated at the extreme end of the cantonment opposite the principal entrance to the reservation. The wards of the hospital itself together with the essential accessory buildings such as the quarters and barracks for officers, nurses, and enlisted personnel, as well as the drill and recreation field, occupy an area of approximately 2½ square miles; this section is separated from the nearest barracks for troops of the line by a distance of about ½ mile. The normal capacity of the hospital is 2000 patients which in a time of emergency has been trebled. At the time of these investigations approximately 1000 troops of the enlisted personnel were assigned to the base hospital. Volunteers were obtained largely from this group of enlisted men since the majority of the medical officers had already been exposed to measles; a considerable proportion of these men had not come in contact with patients, being assigned to duty in the barracks and quarters, in the offices and on fatigue work out of doors. Conditions at Camp Meade were fundamentally identical with those at Camp Devens. The work was transferred to Camp Meade, partly for the purpose of obtaining volunteers from the more isolated rural communities, the majority of the volunteers at Camp Devens having come from the smaller towns.

Precautions Concerning Volunteers.—In the acceptance of volunteers for inoculation with measles there are two cardinal

factors which outweigh all other considerations, namely: (1) the general health of the individual with special consideration of the respiratory tract and (2) the assurance that the adult in question has never had a recognizable attack of measles.

Under ordinary circumstances the virus of measles *per se* does not produce serious results in normal individuals; its disastrous effects arise from the secondary infections. The most important of these are the pneumonias especially those from the pneumococcus, the tubercle bacillus and the hæmolytic streptococcus as in the winter of 1917-18. In addition to thorough physical examination, special attention was given to the points indicated in the following outline in order to secure the fullest possible protection for the volunteers:

I. Bacteriological cultures of throats (several examinations at intervals of three to four days) for,
   1. Hæmolytic streptococcus.
   2. Pneumococcus (any type).
   3. Meningococcus.
   4. Diphtheria bacilli.
   5. Pfeiffer bacillus.

II. Precautions against tuberculosis,
   1. History.
   2. Physical examination.
   3. X-ray findings.

III. Anatomical examination of,
   1. Tonsils.
   2. Sinuses.
   3. Middle ear.

IV. Special isolation for acceptable individuals in order to protect them from intercurrent infections and from spontaneous infection with measles.

V. Special nursing by persons of long experience with measles for any individuals contracting the disease.

If a prospective volunteer showed any of the preceding organisms in the cultural examination of the throat and nasopharynx he was at once eliminated from consideration regardless of whether the organism in question subsequently disappeared. A comparatively large proportion of individuals proved to be unsuitable either on account of the presence of the hæmolytic streptococcus or the pneumococcus. Only a few were rejected on account of a suspected latent tuberculosis. The anatomical examination of the tonsils seemed distinctly important. Individuals who had had an infection of the tonsils at all recently were not considered because, especially under these circumstances, cultural examination of the surface of the gland gives little information concerning the flora contained within the tonsillar crypts. In view of the difficulty of rigidly excluding the presence of the hæmolytic streptococcus by bacteriologic examinations it seemed much safer to conduct these inoculations at a time when infections with this micro-organism were not prevalent in camp. As a practical test of the significance of cultural examinations, Levy and Alexander[1] have noted a very low incidence of complications due to the hæmolytic streptococcus in measles cases whose throat cultures were negative for this organism.

Proper isolation possesses a two-fold importance. In the first place it was very necessary that volunteers found free of pathogenic micro-organisms should be protected from accidental contamination, especially by carriers; secondly, it is of course essential to provide against the possibility of spontaneous infection with measles. With these objects in view, the acceptable volunteers were segregated in a ward removed as far as circumstances would permit from the wards reserved for cases of measles, a distance of about ½ mile. Satisfactory isolation over the rather long period of these experiments was rendered comparatively simple by the discipline developed in military life and by the faithful cooperation of the volunteers themselves. The attendants on the ward for the volunteers were restricted from seeing any patients, but they were not required to isolate themselves. By observing the precautions just outlined, it was felt that the possible dangers inherent in these inoculations would be reduced to a minimum.

Probably the greatest difficulty in these investigations consists in the selection of susceptible adults; in the large cities only a very small proportion of individuals, perhaps 5 per cent, come to adult life without having contracted measles. Age *per se* confers no immunity. Moreover, the disease runs a clinical course which is remarkably true to form. Unrecognized attacks such as abortive cases without a rash constitute, if they occur at all, virtually a negligible factor. The chief difficulty arises from the fact that little or no dependence can be placed on the statement of even the educated adult that he has never had measles. Information was obtained according to the following plan: A census of the detachment was taken and those individuals were at once eliminated who reported having had either measles, "German measles," or scarlet fever.† These three diseases were included on account of possible errors in diagnosis. A limited number were sure that they had had none of these infections; the parents of these men were consulted. At Camp Devens this was done, whenever possible, by the individual himself in person; at Camp Meade a letter was sent to the family of the soldier. Inquiry was made in the first place concerning the individual himself, and secondly, whether he had been exposed to measles through its occurrence in other members of the family. After this information was obtained, an opportunity to volunteer was given to the men in whose cases the replies from the parents were satisfactory, provided that the preliminary bacteriologic examination of the individual was negative. The first cultures were made while the history was being obtained; the final examinations were completed only after the individual had volunteered and had been isolated.

The general plan of the inoculations was designed with the purpose of eliciting the desired information concerning the virus of measles with the production, however, of only an absolutely minimal number of cases of the disease. By employing large series of volunteers, the experimental data could have been obtained rapidly on an extensive scale but only at the cost of

† A detailed report of this census will appear in *The Military Surgeon.*

increased risk to the individuals. With the passing of the military emergency, less attention was given to the time consumed in these experiments, the primary object being to avoid the possible production of measles in man without obtaining results proportionate to the serious responsibility of human experimentation.

PRELIMINARY INOCULATIONS OF HUMAN SUBJECTS

Selection of Measles Cases.—There are two essential criteria which must be met in the selection of measles cases for subinoculation of normal individuals: (1) the patient must be secured in relatively early stages of the disease and (2) he must be free from all other infections, notably syphilis. At the beginning of this work, the collection of blood specimens was purposely delayed until after the rash had appeared; although the blood might, very possibly, be more infective in the pre-eruptive stage, it seemed advisable to duplicate conditions which had previously given positive results in human experimentation.

For the exclusion of syphilis, dependence was placed on a thorough history in all its aspects, clinical examination and the Wassermann test.

Preliminary Inoculations.—The first inoculations were designed to give preliminary information on the following points:

(1) Whether the serum alone, free from red cells, contains the virus of measles.

(2) Whether the organism of measles will pass through a Berkefeld filter.

(3) Whether a series of injections of patient's and convalescent's serum would immunize a human subject without producing an attack of the disease.

(4) Whether a previous attack of measles confers complete immunity or whether a modified attack might result in a partially immune subject with possible attenuation of the virus.

For these inoculations, blood was taken from a moderately severe case of measles 12 hours after the first appearance of the rash. At this time the Koplik spots had already disappeared, the eruption was profuse over the face, back and chest, less intense over the abdomen and only a few scattered spots had appeared on the thighs.

Immediately after collection, one portion of the blood was defibrinated and another was centrifuged for the collection of serum. Part of this serum was mixed with an equal volume of fresh serum obtained from a typical case of measles 10 days after the temperature had returned to normal. Another portion of the original serum was diluted with nine parts of physiological saline; one portion of the diluted serum was kept at room temperature for a control and the remainder was heavily inoculated with *B. prodigiosus* and passed through a Berkefeld filter (so-called N).‡

This was an old filter, much worn, which on several previous tests had successfully held back *B. prodigiosus*. When immersed in water, an additional pressure of 50 mm. of mercury was sufficient to force a fine stream of air through this filter. The time employed in filtering 25 c. c. of the diluted serum was 30 minutes and the pressure was 40 to 50 mm. of mercury; the room temperature was 22° C. Twelve cubic centimeters of the filtrate were inoculated in varying amounts on agar plates and into flasks of broth; the culture media subsequently showed no evidence of growth.

The following subcutaneous injections were made into susceptible individuals: For convenience the susceptible men will be designated by a Roman numeral and the immunes by a letter. One volunteer (I) received 5 c. c. of diluted serum (1-10) unfiltered and kept at room temperature one hour; another (II) received 10 c. c. of filtered serum (1-10 dilution) one hour after collection of the blood; a third (III) received 0.5 c. c. of a mixture of equal parts of patient's and convalescent's sera (undiluted) after incubation for one hour at 37° C.

An immune (A) who had measles 28 years previously received 7 c. c. of defibrinated patient's blood 1½ hours after collection.

None of these four individuals developed any symptoms of measles. Neither did any local changes appear at the site of injection except in the individual who received 7 c. c. of defibrinated blood; in this subject a day after the injection, moderate tenderness on pressure developed accompanied by slight erythema over the injected area. These symptoms persisted for three days.

The chief interest in these negative results centers in the control individual who was expected to contract measles. The delay of one hour before injecting this serum and also the absence of red cells might be regarded as factors contributing toward the negative result. It would appear from previous work that the blood retains its infectivity *in vitro* for at least a day. As regards the red cells, as far as we know, all diseases caused by parasites of the red corpuscles are transmitted by insects whereas droplet infection constitutes the accepted mode of transmission in measles.

The failure of filtered measles serum to infect an apparently susceptible human subject must be borne in mind in connection with the positive result reported by Goldberger and Anderson[4] upon the inoculation of monkeys with filtered serum. These investigators conducted four experiments; in the first two, the results were negative; in the third, one of four monkeys presented slight symptoms only; in the fourth experiment one of two monkeys developed an eruption 21 days after inoculation. These authors conclude that the virus of measles is capable of passing through a Berkefeld candle. No details are given concerning the size of the filter employed or the manner in which the filtration was controlled and conducted.

For the mixture of measles serum and convalescent patient's serum, it was originally intended to conduct a long series of injections gradually eliminating the convalescent patient's serum. It was also intended to hyper-immunize by several intravenous injections of measles serum the immune (A) who failed to develop symptoms after receiving a large quantity of blood subcutaneously. After an interval of one month, a second injection was given consisting of 1 c. c. of serum intravenously. This was collected from a patient 15 hours after the appearance of the rash. No symptoms of any kind resulted from this intravenous inoculation. This work was then sus-

‡ The gradations of Berkefeld filters into coarse, medium and fine (V-N-W) was found frequently to bear little relation to their actual porosity.

pended pending confirmation of the existence of the virus of measles in the circulating blood.

INOCULATION OF SUSCEPTIBLE INDIVIDUALS WITH MEASLES BLOOD

Inoculation of Defibrinated Blood and of Blood Incubated in Ascitic Broth.—The remainder of the work with susceptible individuals has been restricted to an attempt to transmit measles by the injection of patient's blood. Defibrinated blood was injected subcutaneously into two men. Cultures of patient's blood in ascitic broth were inoculated in two other men. This duplication of a method for which successful results have been reported seemed essential in view of the negative result following the inoculation of serum without preliminary incubation. None of these four subjects developed measles; neither did any symptoms appear that could be definitely attributed to the injections. The details are as follows:

Defibrinated blood from an early case of measles, 12 hours after the appearance of the rash, was injected subcutaneously in 2 c. c. quantities in the interscapular area, after a delay of 15 minutes, into two susceptible volunteers. The patient's blood (4 c. c.) without defibrination was added to ascitic broth (50 c. c.) consisting of two parts of meat infusion and 1 part of ascitic fluid. The broth was prepared without the addition of sugar and its reaction to phenolphthalein was equivalent to 1 per cent of normal acid. The ascitic fluid was yellow in color, sp. gr. 1.018 and was free from bile pigments. Repeated aërobic and anaërobic cultures showed no evidence of growth; the ascitic fluid was therefore used without preliminary sterilization. In the culture medium prepared by Hektoen the ascitic fluid was heated at 55° C. for 54 minutes. Differences of an altogether minor nature probably occurred also in the reaction and composition of the broth and ascitic fluid which was employed.

This medium inoculated with the patient's blood showed no evidence of growth after 24 hours' incubation; accordingly, two susceptible men (VI and VII) were inoculated subcutaneously in the interscapular area with 10 c. c. of this mixture of blood in ascitic broth, the mixture being shaken to include corpuscles as well as serum. This quantity represents about 0.2 c. c. of patient's serum.

During the incubation period these men were observed with special reference to:

1. Development of symptoms in the upper respiratory tract.
2. Occurrence of Koplik spots.
3. Body temperature.
4. Total and differential leucocyte count.

A record of the temperature by mouth and of the pulse and respiration was taken as a routine at four-hourly intervals during the day. The entire surface of the body was examined for cutaneous rashes, since, after inoculation, the initial lesions might appear first not necessarily on the face or chest, but on any part of the body. All of these individuals remained essentially free from symptoms.

Inoculation with Skin Lesions.—After an interval of 35 days one of the men injected with defibrinated blood (IV) and another (VII) injected with blood incubated in broth were re-inoculated by smearing the mucous membranes of the eyes, nose and throat with freshly excised morbillous skin lesions. These inoculations possessed a two-fold interest in that a positive result would demonstrate that the virus of the disease is present in the skin lesions and that these individuals who were refractory to an injection of blood were nevertheless susceptible to the disease. No definite symptoms developed. In view of these negative results, the question arose of testing experimentally the susceptibility of these men by inoculating the upper respiratory tract with secretions from the mucous membranes of active cases. The injections of blood, however, had been made from only one case of measles, a single specimen of blood having been taken. Therefore, if the use of nasal secretions should produce measles in one of these subjects, it would only establish that, in a single case of measles, one specimen of blood at a given stage of the disease did not prove infective for a susceptible host. It seemed desirable to defer tests of susceptibility until individuals could be inoculated with blood from several stages of the disease and preferably from more than one patient.

Inoculation of Blood from Pre-Eruptive and Eruptive Stages.—Accordingly for the next step, two susceptibles were inoculated on two successive days with blood taken before and after the appearance of the eruption. Control inoculations were made simultaneously into two immunes. Blood for these injections was secured from two patients; specimens were obtained from one case 30 hours and again six hours before the appearance of the rash and from the other six hours before and 18 hours after the rash appeared. None of these four subjects developed any symptoms of measles.

Of the two susceptibles selected for these injections, one (IX) gave unusually good evidence that he had never contracted measles. This individual had grown up on a farm in West Virginia and had always lived at home. He was 26 years of age being the sixth of 8 children of whom the youngest was 20 years and the eldest 38 years of age. Information was received from the mother and the eldest sister to the effect that measles had never occurred in the household. Then in adult life several older and younger brothers and sisters left home and contracted measles. Of the five older members of the family, the eldest sister contracted measles in 1910; two brothers, the second and third members of the family, developed measles in March, 1917; the fourth member, a brother, has not had measles; a sister, who was the fifth child, developed measles in 1908. Of the two younger children one, a sister, 20 years of age, has never had measles; the other, a brother, 24 years of age, did not develop measles until he enlisted in the army, having contracted the disease at Camp Shelby, Miss., in December, 1917.

On enlisting this volunteer (IX) was assigned to the medical detachment, working exclusively in the officers' quarters. He entered the hospital only on a single occasion, to visit a friend ill with tonsillitis.

In view of the prevalence of measles in the cantonments it might seem that no susceptible individual could escape infection for any length of time. However, there has been a slight but constant incidence of measles at the ports of debarkation even among the troops returning from overseas, notwithstanding the fact that measles has been widely distributed throughout the training camps of this country.

The two immunes were injected in order to test the protection afforded by an attack of measles and also to control

any minor symptoms that might appear in the susceptibles, such as slight changes in the body temperature and in the leucocyte picture. One of these immunes (A) had already been inoculated with blood on two occasions and with secretions from the mucous membranes of patients; the results were negative. The other immune (B) probably had had measles more than 30 years ago and had not previously received any injections in the course of these experiments.

For these inoculations of blood, very favorable cases of measles were obtained during a small epidemic which developed in a country district outside of the camp. Two adult patients were selected. Blood from each of these cases was mixed with 2 per cent sodium citrate in physiological saline; the two specimens of citrated blood were pooled and the mixture was injected into each subject; 24 hours later a second specimen of blood was collected in the same way from each patient and the injections were repeated. Each subject received injections of blood from two patients at two periods of the disease, *i. e.*, the equivalent of four injections in all. On the first day 10 c. c. of blood were taken from each patient, and mixed with an equal volume of citrate solution. On examination, the first patient showed numerous Koplik spots at this time and six hours later the rash appeared. The temperature was 102.8°. The second patient showed only a few isolated Koplik spots and the rash appeared 30 hours later. The temperature was 99°. These two specimens of citrated blood were mixed; each of the two susceptibles (VIII and IX) and the two immunes (A and B) were then injected with 6 c. c. of the resulting mixture, the injection being given in part subcutaneously and in part intramuscularly in the gluteal region. The maximum period of time elapsing between the withdrawal and the injection of the blood was 45 minutes for the first and 15 minutes for the second patient. A duplicate of these injections was made on the next day, 10 c. c. of blood being taken from each case and mixed with 7 c. c. of 2 per cent citrate solution. At this time the Koplik spots had disappeared in the more advanced case; the rash, which had appeared 18 hours previously, was almost confluent over the face, well developed over the chest and very sparse over the abdomen. In the second patient, the Koplik spots were abundant at this time and the rash appeared six hours later. The temperature was 102.2°. After these specimens of citrated blood had been mixed, the same men (VIII, IX, A and B) were injected with 6 c. c. quantities in the same manner in the gluteal region on the opposite side; the time elapsing between the withdrawal and the injection of the blood for the first case, *i. e.*, the one in which the rash had developed, was 40 minutes and for the second case, 10 minutes. These injections were made subcutaneously and intramuscularly in order to duplicate in the first place the manner of inoculation for which successful results had already been reported. It seemed entirely possible that a virus might find better protection for itself in the subcutaneous tissues than when exposed to the fluids and cells of the blood stream. An intravenous injection in addition to those into the tissues was not made in order to determine whether a rash, if one developed, would appear first at the site of inoculation or on the face as in the spontaneous disease. To facilitate these observations the site of inoculation was changed from the interscapular to the gluteal region.

Some slight local reactions followed these injections. At the site of inoculation one of the two susceptibles (VIII) developed well-marked induration appearing eight days after the first injection, persisting very definitely for four days and gradually disappearing during the next 48 hours. In this individual the induration was bi-lateral; in the other (IX) slight induration without any well-defined margin developed at the site of the second injection. This also appeared 8 days after the first injection but persisted for only 48 hours. In one of these men (VIII) a very transient erythema appeared for a few hours over the area of induration, on one side only, on the 10th and again on the 11th day after injection. Neither of the two immunes developed any local induration. One of these immunes (A) on being inoculated in a similar manner 5½ months previously developed a local induration entirely analogous to these two susceptibles. The local reaction, therefore, to the first injection of measles blood varied slightly in the two immunes.

SUSCEPTIBILITY TESTS

When it became apparent that no symptoms of measles would develop from these injections of blood, arrangements were made to test the susceptibility of these men by inoculating the mucous membranes of the eyes, nose and throat with the secretions from the mucous membranes of an early case of measles.

A favorable patient was found for these inoculations. A child, five years of age, the daughter of a physician was exposed to measles by the occurrence of an attack in an older sister. During the incubation period in this child, examinations were made of the nose and throat for pathogenic microorganisms, especially the pneumococcus and the hæmolytic streptococcus. None were found and as far as secondary invaders were concerned, the secretions were regarded as satisfactory. Twelve days after exposure the patient developed a moderate conjunctivitis and coryza accompanied by a temperature of 100°. During the next two days, the temperature was practically normal but on the following morning, 15 days after exposure, it rose to 102.5° with a marked increase in the conjunctival and respiratory symptoms. A few Koplik spots were present. On the following morning these symptoms persisted and profuse eruption appeared over the face spreading gradually downward over the body in the course of the next 48 hours.

Inoculations with secretions from the mucous membranes were carried out on the two susceptibles (VIII and IX), on one of the immunes (A) and on another immune (C) who had not previously received any injections. The latter, a man, 23 years of age, had had measles nine years before. Twenty days after the last injection of blood, these four individuals (VIII, IX, A and C) were inoculated on the mucous membranes of the eyes and nose with the lachrymal secretions and also on the nose and throat with the nasal secretions of the patient. This inoculation was performed on the day of the preeruptive rise in temperature in the child, *i. e.*, 12 days after exposure and four days before the eruption. A similar inoculation was made on the day before the eruption, *i. e.*, on the 15th day after exposure. On this occasion, in addition to duplicating the previous inoculations, the buccal secretions were thoroughly rubbed over the mucous membranes of the mouth and throat of the four subjects. On both days all of these inoculations were made with a sterile cotton swab slightly moistened with physiological saline. This was rubbed rather firmly over the mucous membrane of the patient and then immediately over the mucous membrane of the subject, a fresh swab being used for each inoculation. On both occasions the

four subjects were in the same room with the patient; on the day of the first inoculations, the patient not being confined to the bed was allowed to play with these men three-quarters of an hour.

Intravenous Injection of Blood.—In addition to these inoculations with secretions of the mucous membranes, one more susceptible (X) was inoculated with measles blood. A specimen was taken from this same child within from 6 to 10 hours after the appearance of the rash; 0.5 c. c. was injected subcutaneously and 1.5 c. c. intravenously immediately after withdrawal before the blood had time to clot.

Neither the intravenous injection of blood nor the inoculations of the secretion produced, in these five subjects, any respiratory symptoms or any rash. Only insignificant fluctuations occurred in the temperature and in the total and differential leucocyte count.

For the susceptibility tests in these men it might naturally be expected that control inoculations would have been conducted with a susceptible individual who had not had any previous inoculations of blood. The production of measles in a control case would furnish some additional evidence but certainly would not in any sense afford proof that these men were susceptible and had been immunized by the injections of measles blood.

In drawing conclusions concerning these inoculations of blood and mucous secretions in these susceptible men, there are two unknown factors to be determined, namely:

(1) Whether the individuals in question (VIII and IX) at the conclusion of the inoculations were immune and if so

(2) Whether the immunity was conferred by the injection of blood or acquired possibly through a previous attack of measles.

The results of the susceptibility tests leave little doubt but that these men were immune; they were exposed to natural infection by an early case of measles and they were also thoroughly inoculated artificially. The high degree of communicability characteristic of the disease leaves little room to suppose that these men might be infected by exposure now to a second case. The evidence of susceptibility of one of these volunteers (IX) is certainly much more concrete than the general arguments that can be brought in favor of a previous attack of measles. It would seem distinctly possible that the injection of blood had produced an active immunity.

A priori one would not expect that substantial immunity could be obtained without the production of marked local or general symptoms. However, in the case of varicella, Hess and Unger[5] report active immunization by the intravenous injection of the contents of vesicles without the production of subjective symptoms. It is noteworthy, however, that control individuals inoculated on the mucous membranes failed to develop varicella. Recent work by Richardson and Connor,[6] though affording no final proof, suggests the possibility of obtaining active immunity against measles without the production of definite symptoms.

Lastly it should be emphasized that conclusive demonstration of the non-infectivity of measles blood upon injection into a susceptible individual would still fail to prove the absence of the virus of the disease in the circulating blood. It is very doubtful, for example, whether a susceptible human being would be infected by the injection of a moderate amount of blood of a typhoid patient taken during the stage of bacteriæmia. It is a theoretical possibility that the blood of a measles patient might reproduce the disease when inoculated on the mucous membranes though not upon injection into the body tissues.

For the sake of convenience, the inoculations reported in the preceding pages have been collected in the following table:

TABLE OF INOCULATIONS

Individual	Material	Amount	Mode of Inoculation
Susceptible I.	Blood serum, eruptive stage.	0.5 c.c.	Subcutaneous.
Susceptible II.	Blood serum, eruptive stage, filtered.	0.5 c.c.	Subcutaneous.
Susceptible III.	Blood serum, eruptive stage, incubated with convalescent's serum.	0.25 c.c.	Subcutaneous.
Immune A.	Defibrinated blood, eruptive stage.	7 c.c.	Subcutaneous.
1 month later.	Blood serum, eruptive stage.	1 c.c.	Intravenous.
2½ months after 2d injection.	Mucous secretions, eruptive stage.	.....	On mucous membranes.
2 months after 3d injection.	Citrated blood, pre-eruptive stage.	*3 c.c.	Subcutaneous and intramuscular.
1 day after 4th injection.	Citrated blood, pre-eruptive and eruptive stage.	*3 c.c.	Subcutaneous and intramuscular.
3 weeks after 4th injection.	Mucous secretions, pre-eruptive stage.	.....	On mucous membranes.
3½ weeks after 4th injection.	Mucous secretions, pre-eruptive stage.	.....	On mucous membranes.
Susceptible IV.	Defibrinated blood, eruptive stage.	2 c.c.	Subcutaneous.
5 weeks later.	Rubeolous skin lesion.	.....	On mucous membrane.
Susceptible V.	Defibrinated blood, eruptive stage.	2 c.c.	Subcutaneous.
Susceptible VI.	Blood, eruptive stage, incubated in broth.	*0.4 c.c.	Subcutaneous.
Susceptible VII.	Blood, eruptive stage, incubated in broth.	*0.4 c.c.	Subcutaneous.
5 weeks later.	Rubeolous skin lesion.	.....	On mucous membrane.
Susceptible VIII.	Citrated blood of two patients, pre-eruptive stage.	*3 c.c.	Subcutaneous and intramuscular.
1 day later.	Citrated blood of two patients, pre-eruptive and eruptive stages.	*3 c.c.	Subcutaneous and intramuscular.
3 weeks later.	Mucous secretions, pre-eruptive stage.	.....	On mucous membrane.
3½ weeks later.	Mucous secretions, pre-eruptive stage.	.....	On mucous membrane.
Susceptible IX.	Duplicate of 4 inoculations of Susceptible VIII.		
Susceptible X.	Whole blood, eruptive stage.	2 c.c.	Intravenous and subcutaneous.
Immune B.	Duplicate of the first and second injections of VIII and IX.		
Immune C.	Duplicate of the third and fourth injections of VIII and IX.		

*Calculated amount.

CONTROL INOCULATIONS

Injection of Ascitic Broth and Normal Blood.—For the intensive inoculation of susceptible individuals with measles blood it might seem only natural to have included the injection of measles blood incubated in ascitic broth in addition to the other injections. A control series of injections, however, had already shown that normal blood in ascitic broth was not altogether free from toxic action.

The same lot of broth and ascitic fluid already described for the incubation of measles blood was mixed with normal human blood

(4 c. c. to 50 c. c. of ascitic broth) and incubated for 24 hours. Quantities of 5 to 7 c. c. were injected subcutaneously into 10 individuals; six remained practically free from symptoms. Of the other four, one immediately after injection maintained a temperature of 99° to 99.5° for nine days; another, six days after injection, developed a temperature of 100.2° accompanied by a few large papules over the forehead and a moderate degree of inflammation of the pharynx; the two others, nine days after injection, developed temperatures of 100° and 101° with definite pharyngeal symptoms but unaccompanied by any rash.

In a second group of controls with the same technique but with another lot of broth and of ascitic fluid, the reactions were similar but rather less marked. Of 10 individuals, three remained entirely without symptoms. The results in the others were as follows: in two cases, 10 days after injection, the maximum temperature in one was 99.8° and in the other 100.2°; three other cases reacted much earlier, the temperature in one on the fifth day being 100° and in the other two 99.4° on the sixth day. One case presented a slight rise in temperature on several days as follows, 99.2° on the fourth and fifth days, 99.4° on the sixth day. On these days, this man also developed a fairly extensive maculopapular eruption appearing first over the chest and then over the arms. When his temperature returned to normal the rash faded leaving pigmented areas which gradually desquamated. Another subject on the third, fourth and fifth days after injection developed a well-marked erythema which was widely distributed over the chest, back, abdomen and extremities. The temperature continued normal, except on the 13th day when it rose to 99.4°. In this second group of controls the leucocytes were counted whenever a rise in temperature occurred, but no leucopœnias were observed.

These reactions, though very mild, indicate the advisability of conducting control inoculations when similar injections are performed to test for the presence of a virus; this is especially true in view of the difficulty of duplicating exactly a rather complex culture medium. Although these symptoms did not simulate measles in any way, nevertheless the interpretation of atypical temperature curves or rashes might be confusing; the detection of a pre-eruptive rise in temperature might be very difficult when complicated by the possible effects of ascitic broth.

CLINICAL EVIDENCE

Intra-uterine Infection.—Medical literature, especially the text-books, frequently contain the statement that pregnant mothers, having contracted measles, occasionally give birth to a child with a fully developed rash. These reports of intra-uterine infection do not bear complete evidence of authenticity. In many instances, satisfactory evidence is lacking for the diagnosis of measles especially in the child; in other cases, the period elapsing after birth before the rash appears is sufficiently long to permit of extra-uterine infection. Although the cases are comparatively rare, it appears, on the other hand, that a pregnant mother developing measles at term does not ordinarily infect the child *in utero*. Lastly, the development of measles in a new-born child would not harmonize with the view that infants during the first three months of life are immune from the disease. This apparent discrepancy might be explained by Herrman's [9] statement that children of susceptible mothers are not immune from measles, a view, however, which is not supported by the natural immunity of infants to some other acute infections, notably scarlet fever and diphtheria.

Characteristics of the Eruption.—The question whether the virus of measles gains access to the circulating blood requires for a completely satisfactory answer the explanation of a striking clinical symptom. *A priori* the remarkably constant appearance of the rash widely spread over the body would seem obviously to point to the distribution of the virus by the blood-stream. Unlike the rashes of other infections, of serum disease and of the food and drug intoxications, the eruption of measles always appears first on the upper part of the body, commonly on the face, from where it spreads steadily downward over the trunk and arms reaching the lower extremities on the second or third day. This phenomenon is very striking and very constant. It has been recognized as one of the distinguishing features of the eruption of measles. The average period for this progress over the body is usually stated as two or three days with extreme limits of one to five days. In a fairly definite but less striking manner the eruption fades in the order of its appearance; thus the rash often almost disappears on the face before it is fully developed on the feet. Virtually no explanation has been suggested for this characteristic progress of the skin eruption. Von Pirquet,[10] assuming that the virus is present in the blood stream, advances the view that the rash is a manifestation of allergie and that the order of appearance of the skin lesions is directly proportional to their distance from the heart and great vessels. In support of this view, figures are given in detail of the distance from the skin lesions by way of the arteries to the heart. It is very difficult to conceive of any explanation that is consistent with the mechanical features of the circulatory system. Moreover, other exanthems which certainly are distributed by the blood-stream do not progress in this manner over the body. A notable example is small-pox; the portal of entry is probably similar to that of measles, but one commonly examines the palms for the first nodules. Von Pirquet departs widely from the accepted views in considering that the rash of small-pox resembles measles in its origin and progress over the body.

Possibly the lymphatic system offers a more satisfactory basis than the circulating blood for the explanation both of the usual point of origin of the rash and its subsequent distribution. The mucous membrane of the upper respiratory tract and of the eyes may safely be regarded as the portal of entry of the virus of measles and the seat of the primary lesion. Notwithstanding some variation in individual cases as well as some minor differences of opinion, the skin lesions usually appear first on the face. It is conceivable that the virus travels by way of the lymphatics not unlike the primary infection of the nasal mucous membrane in leprosy with the subsequent involvement of the skin of the face. The gradual progress of the measles rash has certain features in common with the spread of erysipelas, an infection which travels by way of the lymphatics. The plexus of lymphatics forming a complete network over the body would furnish anatomical communications consistent with the distribution of the rash. The valves of the lymphatic system which normally direct the flow of lymph in certain definite channels would not necessarily constitute an effective barrier against the spread of an infec-

tious agent; a rapidly multiplying virus could conceivably grow against this feeble current of lymph and also grow through the delicate valves.

The preceding discussion has been based on the hypothesis that the morbillous exanthem is produced, not by an extracellular toxin but by the action of the virus of the disease itself, in a manner analogous to what occurs in small-pox and chicken-pox. A toxin could hardly distribute itself either by the blood stream or by the lymphatics in a manner that would explain the symptoms observed in measles. The virus of the disease, if it is distributed by the lymphatics, would almost inevitably be swept into the blood stream where it might readily persist temporarily even though it did not multiply. The clinical evidence, however, can only be regarded as suggestive; experimental proof must be obtained before any final decision is permissible. The ultimate explanation of the progress of the rash of measles will be of distinct interest and value.

DISCUSSION OF RESULTS

There is, unfortunately, a striking discrepancy between the negative results following these inoculations of measles blood and the successful experiments previously reported under essentially similar conditions. A careful examination of the data recorded in this paper has failed to suggest any correlation of these diametrically opposite results. Eight apparently susceptible individuals have been inoculated with blood in various ways but none developed measles. In the selection of measles patients for these inoculations, the diagnosis both of the disease itself, especially during an epidemic, and also the stage of the disease offers little difficulty. In any negative results in adults the crucial factor is the question of susceptibility. While some of these eight individuals may in reality have been immune, it seems that, at the least, several of them must have been susceptible. As already suggested, the two who received intensive inoculations of measles blood and responded negatively to susceptibility tests may have been immunized by the injections; if one is not willing to consider the possibility of immunization there still remain six other cases requiring explanation.

There are certain points of especial interest concerning the two experimental cases reported by Hektoen. The extremely small quantity of serum, approximately 0.1 c. c., employed by this author constituted one of the significant features of his experiment. A few but not many infections can be readily reproduced by the injection of minimal quantities of blood. In cattle plague as little as 1/60 c. c. of blood may prove infective;[11] Marchoux, Salimbeni and Simond[12] produced yellow fever with 0.1 c. c. of serum.

It will be recalled that these successful experiments with measles were conducted prior to our knowledge of serum sickness; however, only homologous proteins were employed and typical serum-disease can therefore be eliminated. In the control tests reported in this paper the injection of mixtures of normal blood, ascitic fluid and broth produced a slight temperature and rash in occasional instances, but in no case could the symptoms have been confused clinically with measles.

Spontaneous Infections.—Sporadic cases of measles may develop so insidiously that even with careful isolation, the possibility of spontaneous infection during experimental inoculations must be borne in mind. A typical illustration occurred at the base hospital at Camp Devens, Mass. A patient had been in the general wards of the hospital for two months; during this period it was not possible to trace any contact with a case of measles. He was then transferred to the psychopathic ward where, after thorough isolation for one month he developed a typical attack of measles. The temperature chart showed a characteristic pre-eruptive rise accompanied by conjunctivitis and coryza. The symptoms increased; Koplik spots appeared, the temperature rose again, a characteristic rash developed followed by the usual pigmentation. It was not possible to locate any third person who might have carried the infection to this patient. Such examples are, however, so rare that it is by no means plausible that both of Hektoen's cases could be explained in this manner. This is especially true in view of the fact that the experimental subjects in question underwent a period of isolation much longer than the normal incubation time of the disease before any injections were made and they were subsequently carefully protected from spontaneous exposure.

The diagnosis of these experimental cases was based essentially on the rise in temperature after a typical period of incubation and the development of a definite rash. In both patients the rash started on the face. In one case the progress of the rash over the body is described; its distribution was almost complete in about five hours. This period would be extremely short for spontaneous measles, being much more characteristic of rubella. Information concerning the lymphatic glands in these patients would have been of some value in differentiating these two diseases. Although the injections were made over the chest and over the back it is of interest to note that the rash appeared first on the face just as when the virus enters the body by way of the mucous membrane. In one of these cases a typical bran-like desquamation is described.

Respiratory symptoms were absent in one of these cases, a feature which has led Ustvedt[13] to suggest the possibility of doubt concerning the diagnosis of measles. However, it is not at all surprising that the mucous membrane of the respiratory tract should escape involvement when one considers that the portal of entry and very possibly the seat of the primary lesion was changed from the mucous membranes to the body tissues. Such absence of respiratory involvement in *measles inoculata* would constitute a hopeful point in securing active immunization without running the risk of pulmonary complications. Likewise, from the standpoint of protective inoculation, it is encouraging to note that evidence of malaise was absent altogether in one patient and very mild in the other.

It is noteworthy that the charts in neither case exhibit any pre-eruptive rise in temperature, a characteristic which though very common is not constant in spontaneous infections and it is certainly not an essential criterion for the diagnosis of

measles inoculata. It is desirable that certain deficiencies concerning these cases should be supplied, more especially the observations concerning the leucocyte count and the occurrence of Koplik spots. This information would be helpful in the differential diagnosis.

In some respects, therefore, these experimental cases differed from the usual course of spontaneous measles notably in the rapidity of the spread of the rash, in the absence of a pre-eruptive rise in temperature, in the absence of respiratory symptoms in one case and of malaise in the other. Such modifications, however, are of an essentially minor nature and might readily be explained as the result of the subcutaneous injection as contrasted with spontaneous infection. The occurrence of such modifications in the disease, however, would require thorough confirmation.

General Considerations.—Some of the infectious diseases of man can be readily transmitted from infected to susceptible individuals by the injection of blood; such transmission has been fully demonstrated for yellow fever,[12] malaria[14] and trench fever.[15] According to the conclusions of Hektoen this same statement would also apply to measles. To appreciate fully its importance, it must be clearly borne in mind that for trench fever, malaria and yellow fever the natural portal of entry is through the skin; in measles the primary lesion occurs in the mucous membrane. By radically altering the portal of entry or the seat of the primary lesion of a given virus remarkable changes may be produced in its effect even on a highly susceptible host; the vibrio of Asiatic cholera produces infection when ingested by way of the alimentary tract but relatively large amounts of living and virulent cultures can be injected subcutaneously with impunity.

After noting certain definite exceptions it would seem possible to develop some general rules concerning fundamental principles involved in the artificial immunization against any given infection. Precise data, from the very nature of the subject, are not available on account of the difficulty of obtaining information concerning the infectivity of pathogenic micro-organisms for man. The subject may be conveniently considered under three headings:

1. *For those infections in which the mucous membrane constitutes the portal of entry and in which the classical lesions of the disease are in general limited to the tissues accessible by direct continuity, the causative organism may in many instances be injected subcutaneously in a viable condition in moderate dosage with impunity.*

In the case of bacillary dysentery and Asiatic cholera, living attenuated cultures of the causative organism have been used freely by subcutaneous injection as a vaccine. For the pneumococcus, Cecil and Blake,§ at the Army Medical School, have recently demonstrated in monkeys (*Macacus syrictus*) that as little as one-millionth of a cubic centimeter of a broth culture may produce a fatal pneumonia upon intratracheal injection. The subcutaneous injection of one-thousandth of a cubic centimeter of the same culture may or may not produce a septicæmia. In either case, those animals which recover possess a distinct immunity. In the case of *B. diphtheriæ* virulent strains growing on a wound usually produce no symptoms in man. From the clinical evidence one would not expect that the hypothetical organisms of whooping cough and of influenza would produce an infection upon subcutaneous injection.

2. *As a corollary to the first statement, when the natural portal of entry of a pathogenic organism occurs through the skin, then artificial injection into the tissues or blood stream causes an infection which reproduces the typical manifestation of the spontaneous disease.*

Of the more important diseases in which the portal of entry occurs through the skin we may consider bubonic plague, tetanus, glanders, anthrax, malaria, trypanosomiasis, hydrophobia, kala azar, relapsing fever, typhus fever, yellow fever, dengue fever, trench fever and the spotted fever of the Rocky Mountains. In the case of plague, malaria, yellow fever, trench fever, typhus and probably dengue, it has been demonstrated experimentally that the subcutaneous or intramuscular injection of the virus, in virulent form, reproduces the typical features of the spontaneous disease; a similar result could be confidently expected in the remainder of the diseases just mentioned.

The disease, plague, occupies a position of an intermediate type which is unique. *B. pestis* exhibits the characteristic of possessing two distinct portals of entry resulting in the production of two clinical types of disease, the pneumonic and the bubonic form of plague. If insect transmission of *B. pestis* were unknown, bubonic plague would not occur spontaneously; experimentally it could be designated appropriately as *plague inoculata* inasmuch as it differs markedly from the pneumonic form.

3. *For those infections in which the virus enters by way of the mucous membrane and gives rise to metastatic lesions in other tissues of the body, the injection of the virus into the body tissues usually produces an infection; sometimes the essential clinical characteristics of the disease are duplicated, but in other instances a modified infection results quite unlike the spontaneous disease.*

The first and third groups must be looked upon merely as extremes between which there can be no sharp dividing line; for instance, typhoid fever constitutes a somewhat intermediate example. Although *B. typhosus* can infect in moderately small numbers by way of the mucous membrane, nevertheless, living cultures can be injected subcutaneously with the production of nothing more than a local reaction.

The meningococcus, *M. melitensis, Treponema pallidum* and the causative organism of poliomyelitis may conveniently be considered together. Injection of monkeys with the virus of poliomyelitis and the accidental injection of man with *T. pallidum* have produced typical infections; similar results could probably be obtained with the meningococcus and with *M. melitensis,* two organisms which produce little or no local lesion on their passage through the mucous membrane.

Small-pox and chicken-pox present important conditions for consideration. When the virus of small-pox is inoculated

§ Personal communication.

on the skin instead of on the mucous membranes, *small-pox inoculata* develops and the disease, as it occurs spontaneously, cannot be reproduced in this manner. According to Hess and Unger[9] the virus of chicken-pox, when injected intravenously, gives rise to no symptoms but apparently reproduces some immunity.

Exceptional conditions occur in two of the diseases of lower animals. In cattle plague extensive primary lesions occur on the mucous membrane of the intestinal tract and eventually a septicæmia develops. Experimentally the virus behaves in an almost unique manner in that small doses of blood injected subcutaneously set up a fatal infection. Besson[10] states that the influence of the site of injection of the bacillus of symptomatic anthrax is very marked. A dose of the virus which will kill an ox on injection into the cellular tissues of the body will produce merely a benign infection when injected into the connective tissues of the neck, tail or ear and is followed by permanent immunity.

In the preceding discussion the objection may at once be raised that the normal healthy adult can readily withstand the inoculation of a few living and fully virulent pathogenic organisms. A distinct step would be gained if it can be shown that those organisms which normally enter the body through the skin can, as far as injections into the tissues are concerned, set up an infection from the introduction of much smaller numbers than in the case of those micro-organisms which first infect the mucous membranes. In attempting prophylactic immunization against a given disease it is fundamentally necessary to consider the natural portal of entry of the virus in question.

RESUME

An effort has been made to determine whether the virus of measles exists in the circulating blood of a patient, permitting the transfer of the disease from man to man by the inoculation of blood. The prominent data bearing directly on this subject are:

(1) The successful transmission in two cases previously reported by Hektoen,

(2) The failure in eight instances recorded in this paper to transmit measles by the injection of blood,

(3) The clinical phenomenon of the origin of the rash on the face or upper part of the body with its gradual progress downward requiring from one to three days to complete this march.

The symptoms of the two experimental cases that have been reported were not entirely characteristic of the classical type of measles. Although the disease varies extremely little in its clinical manifestations, distinct modifications might readily occur under experimental conditions.

As regards the negative results obtained in these eight individuals, the principle difficulty arises in establishing, by the clinical history, the susceptibility of an adult to a disease so generally prevalent as measles. In some of the men who were inoculated the circumstantial evidence of susceptibility was unusually strong.

Failure to transmit the disease by the injection of blood does not preclude the existence of the virus in the blood stream even in moderate amount. There is some evidence that these men not only failed to become infected but that they were actively immunized by the injection of blood; such an assumption would, of course, presuppose the existence of the virus in the blood stream. Indeed the agent which excites the rash might readily gain access at least temporarily to the blood-stream regardless of whether its distribution takes place by the lymphatics or by the circulating blood.

The constant origin of the eruption on the upper part of the body and its gradual and orderly extension downward is quite unlike the development of eruptions in which the virus is known to be distributed by the circulating blood.

Measles originates as an infection of the respiratory and conjunctival mucous membranes and the virus does not set up metastatic infections in the viscera of the body in the spontaneous disease. Failure to reproduce the disease by subcutaneous injection of the virus is in keeping with the behavior of several other pathogenic micro-organisms of the respiratory and gastro-intestinal tract.

The virus of measles *per se* does not produce serious effects, but one attack of the disease gives rise to a well-marked immunity. With appropriate isolation of the virus it would seem that a substantial active immunity should be obtainable with a minimum of inconvenience to the individual.

CONCLUSIONS

1. The question of the transfer of measles from man to man by the injection of a patient's blood is entirely reopened by the eight successive negative inoculations recorded in this paper.

2. The failure to transmit the disease in this manner does not necessarily exclude the possibility of the occurrence of the virus of the disease in the circulating blood; some evidence was obtained indicating the possibility of producing active immunity by the injection of patient's blood.

This experimental study of measles by the inoculation of volunteers was authorized by General Gorgas and General Ireland at the request of Colonel Frederick F. Russell. The officers and men volunteering for this work signed the following statement:

"I hereby volunteer as a subject for inoculation with measles in order to promote the work undertaken in the United States Army for securing a protective inoculation against this disease."

Having seen the serious consequences of measles in camp life, these men, for no reward to themselves, gladly accepted the risk inherent in these inoculations simply from a desire to be of service. Throughout this work they co-operated cheerfully and conscientiously in carrying out the long isolation essential during these investigations.

The Surgeon General's recognition of their services was made by Colonel Russell in the following letter:

"The Surgeon General has been informed of the fact that you volunteered for the measles investigation. He desires me to express to you his appreciation of the patriotism and devotion to duty that you have shown and to assure you that your contribution to the cause is appreciated by him just as much as was the bravery of the men who went into the fight in France."

It is a pleasure to acknowledge the constant co-operation of Colonel Frederick F. Russell throughout these investigations. I

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THE RÔLE OF THE X-RAY IN THE DIAGNOSIS OF LONG-STANDING RENAL TUBERCULOSIS

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The progress which has been made in the last decade in the investigation of diseases of the urinary tract, thanks to the use of the cystoscope and ureteral catheter, has made possible a differentiation of lesions which before that time had been unrecognizable.

There is no disease of the kidney which can be more certainly diagnosed by the use of these modern methods than renal tuberculosis. The demonstration of diminution in the renal function, together with the presence of pus cells and of tubercle bacilli in the catheterized specimen of urine collected from one side, leaves no possibility of doubt as to the condition with which one has to deal.

In the majority of cases a diagnosis is possible by the use of these methods. But in many cases of renal tuberculosis, especially those of long duration, cystoscopy and ureteral catherization are impossible on account of the changes which have taken place in the bladder mucosa. It may be impossible to recognize the ureteral orifices or they may not admit the ureteral catheter; and there are also some cases in which extensive ulcerative processes have led to such contracture of the bladder as to make the introduction of the cystoscope impossible or too painful for the patient to bear. In many of these cases, although tubercle bacilli may be demonstrable in the bladder urine, owing to the fact that the ureters cannot be catheterized, it is impossible to determine in which kidney the lesion is located, because it must be remembered that in most cases of renal tuberculosis there are a few definite symptoms referred to the kidney itself.

When it is possible to introduce the ureteral catheter, the diagnosis is usually easy, but there are types of long-standing chronic tuberculous processes with sluggish ulceration in which only a few pus cells can be demonstrated and tubercle bacilli may not be found even in repeated catheterized specimens.

In some cases in which the disease has progressed to complete destruction of the kidney terminating in caseation and deposition of calcium salts—the so-called auto-nephrectomy—the deposition of calcium salts will cast a shadow on the x-ray negative varying in density in proportion to the extent of the process. It is in just these cases where for any of the above reasons a definite diagnosis cannot be made that the x-ray will often be the determining factor in the recognition of the lesion.

When the normal kidney substance has been largely replaced by calcified caseation, a complete outline of the kidney and even the ureter may be seen on the x-ray plate and in such cases a definite diagnosis can be made immediately without the demonstration of tubercle bacilli and without recourse to ureteral catheterization except to determine the integrity of the opposite kidney.

It should, therefore, be a routine procedure to precede cystoscopy and ureteral catherization by plain x-ray examination of the urinary system in all suspected cases of renal tuberculosis.

When the calcification is limited to a single area in the kidney there may be some difficulty in differentiating this process from calculus, but a critical observation of the